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UNIVERSITY OF TEXAS MEDICAL BRANCH
Galveston, Texas
January 12, 1953

In reply to:
CNR:442:LAS:cvj
Ser 31159
19 December 1952

Dr. Leo A. Shinn, Head
Biochemistry Branch
Office of Naval Research
Department of the Navy
Washington 25, D. C.

Dear Doctor Shinn:

The Semi-Annual Report of Progress under ONR grant 553-(00)-NR-120-178 for the period July 1 - December 31, 1952 is as follows:

a) Scientific Progress. 1. Further behavior studies of Sprague-Dawley rats have included tests of relearning a multiple-T maze after some 100 days of voluntary intake of 10% alcohol. This study was reported by W. T. Moore to the Southwestern section of the Society of Experimental Biology and Medicine, and indicates that rats having a high voluntary preference for alcohol relearn a maze rapidly; those with a low preference for alcohol did poorly, probably because of high incidence of a respiratory infection in this group. 2. Aggressivity tests indicate that rats having a high preference for alcohol are more aggressive. It has not yet been possible to complete a proposed analysis of the matrix of various types of behavior tests in relation to voluntary alcohol intake of individual rats. Considerable thought and work has been devoted to simplifying these tests so that a battery of suitable tests may be given within a life-span. 3. Additional drugs which have been given to rats during voluntary intake of 10% alcohol include the sulfonamides, penicillin Na and isonicotinyl hydrazide. The sulfonamides and penicillin were given to healthy rats and to rats suffering a severe pneumonitis new to our colony. This infection was characterized by consolidation of discrete areas of the lungs, suggesting a bronchogenic or peribronchial origin. Professor F. B. Engley, Jr., of our Department of Bacteriology and Parasitology, considers this to represent a Corynebacterium pseudotuberculosis infection, but the pathogen was not isolated. Nearly all affected rats had an initially low preference for alcohol. The infection has now been eradicated from our colony. Neither the infection nor the drug treatment had any large effect on voluntary alcohol intake. Isonicotinyl hydrazide had only minor effects when given to healthy rats on a diet deficient in the vitamin B complex. Further work will be done with sulfonamides and isonicotinyl hydrazide on healthy rats, since the metabolic and central nervous system effects of these agents are of interest. Preliminary studies of isonicotinyl hydrazide were reported by R. G. Brown to the Madison meeting of the American Society for Pharmacology and Experimental Therapeutics, and will be published as an abstract in the December, 1952, issue of the Journal of Pharmacology and Experimental Therapeutics (see appended abstract, 1).

4. Dr. Melvin Schadewald has completed histologic studies on the olfactory tractotomies done in the series of rats studied as to effects of anosmia on preference for alcohol. These data, together with our studies with $ZnSO_4$ and K tellurite, will be published, by invitation, in the Loewi Festschrift issue of the Arch. exptl. Pathol. und Pharmakol. The manuscript will be submitted as a Technical Report at the time it is sent to the publisher.

5. Studies on species variation in voluntary intake of alcohol have been further expanded by Dr. Schadewald, who has found the C_3H strain of mice to be highly irregular even on a stock diet, and Orizomys, the rice rat, to be regularly very low in its preference for alcohol. Work with other species has been continued, to obtain records over at least 12 months as reference data, for Sprague-Dawley rats, cotton rats, deer mice and hamsters. Wild Texas cotton rats are now available in our laboratory, and will be bred and used for comparison of their alcohol preference with that of Sigmodon h. hispidus. (See appended abstract, 2, which will appear in the December, 1952, issue of the Journal of Pharmacology and Experimental Therapeutics).

6. A study of effects of pregnancy and lactation on voluntary intake of 10% alcohol by hamsters now includes data on over 50 productive matings and a like number of unproductive matings as controls. This will be reported by J. W. Carver to the Society for Pharmacology and Experimental Therapeutics in April, 1953, and will appear in Federation Proceedings as an abstract (see appended abstract, 3). A study of diurnal and daily weight changes in relation to posology of voluntary intake of alcohol in hamsters will be reported by C. D. Chamberlain, Jr., at the same meeting (see appended abstract, 4).

7. Rats with very high and very low preferences for alcohol will be tested in regard to function of the liver, and the effect of labile methyl donors on alcohol preference and liver function will be tested by a technic perfected by J. B. Nash, L. Gutiérrez Vela and others in this laboratory. This study will be reported by P. Y. Wong to the Society for Pharmacology and Experimental Therapeutics, in April, 1953 (see appended abstract, 5).

8. A study of sex variation in preference for alcohol, suggested by Dr. Louis Levin, has been extended to a study of results of gonadectomy in high-preference male rats and low-preference females. This supplements work previously noted on effects of heroic doses of progesterone and estradiol benzoate in male and female Peromyscus c. californicus. This work will be reported by Dr. Schadewald to the Society for Pharmacology and Experimental Therapeutics in April, 1953 (see appended abstract, 6).

9. Genetic factors in preference for alcohol are being studied further, in regard to alcohol preference of the progeny of hamsters and cotton rats of known alcohol preference. Conclusions at this time would be premature.

10. Numerous data are available on effects of segregation and grouping of animals on their voluntary intake of alcohol. These have been gathered incidentally to other studies, such as in breeding experiments, housing of litters together for some time after weaning, and other phases primarily significant to other parts of the study. It is planned that these data will be supplemented by short-term segregation and grouping of large numbers of rats and of other species.

11. The effect of environmental temperature on alcohol preference was noted long ago by Mardones and Onfray (Rev. Chilena Hig., 4: 293, 1942) to be so great that concomitant controls are always necessary. No definitive study has appeared; it has still been impossible to add more than casual observations to our suggestive previous studies. Until administrative interest is recruited in obtaining reasonably reliable use of the excellent air-conditioning facilities potentially available to us, this significant part of the study cannot be completed.

12. A study which shows that a diet containing yeast autoclaved at pH 9, as used by Williams et al., contains no specific factors causing increased alcohol consumption has been completed. This study will shortly be submitted for publication in Texas Reports on Biology and Medicine.
13. Further studies on effects of liver, spleen and yeast supplements have been compared with effects of B-complex supplements. This work may clarify some of the current concepts of the significance of the B-complex and of linolenic or linoleic acid. A part of this work is to be reported by J. B. Nash to the Society of Pharmacology and Experimental Therapeutics, in April, 1953 (see appended abstract, 7). Feeding with single fatty acids from C_6-C_{16} apparently has little effect on alcohol preference of rats fed a diet deficient in the vitamin B complex, plus 5% of these acids. Many data in this and our previous reports may be taken to indicate that a partial inanition, brought about by deficient diets, may be reflected in increased voluntary intake of alcohol in a more or less isocaloric compensation for lack of nutrient intake as food; it might follow that the supposedly specific effect of the vitamin B complex is due in large part to a correction of the anorexia provoked by a repulsive diet. Tests are in progress in which supra-optimal amounts of B-complex vitamins are given to rats during partial and complete inanition.
14. Twenty-eight rats will be used in a preliminary test of effects of adrenalectomy on alcohol preference; these rats have been studied for approximately 110 days in regard to daily observations of their alcohol and water intake. It is hoped that these rats, as well as gonadectomized rats, will be particularly suitable for testing effects of single steroids and other agents affecting metabolism.

b) Change in emphasis. The "considerable change in emphasis of the work" noted in our last report persists, since the lack of proper functioning of air-conditioning facilities demands delay of valid study of several of the more significant problems for which the grant was made. Very little in the way of work or expense is required to adjust the air-conditioning apparatus of the Gail Borden Building.

c) Bibliography:

Brown, Robert G. and G. A. Emerson: Effects of isonicotinyl hydrazine on smooth muscle. (Presented at the meeting of the American Soc. Pharmacology and Experimental Therapeutics in Madison, Sept. 1952) Abstract to be published in the December, 1952, issue of the Journal of Pharmacology and Experimental Therapeutics.

Emerson, G. A., R. G. Brown, Joe B. Nash and W. T. Moore: Species variation in preference for alcohol and in effects of diet or drugs on this preference. (Presented at the Madison meeting in Sept. 1952). Abstract to be published in December, 1952, J. Pharmacol. and Exptl. Therap.

Nash, Joe B., W. T. Moore and G. A. Emerson: Effects of sulfonamides on voluntary choice of 10% V/V alcohol by albino rats. (Read by title at the Madison meeting in Sept. 1952). To be published in December, 1952, J. Pharmacol. and Exptl. Therap.

Carver, Joe W., Joe B. Nash, G. A. Emerson, W. T. Moore: Effects of pregnancy and lactation on voluntary alcohol intake of hamsters.*

Chamberlain, Charles D., Jr., C. D. Leake, G. A. Emerson: The dose-effect relation: critique of estimation of mass of biological material.*

Wong, Peter Y., C. D. Chamberlain, Jr., M. Schadewald, Joe B. Nash, G. A. Emerson: Sulfobromophthalein clearance in white rats after damage to the liver.*

Schadewald, Melvin, G. A. Emerson, W. T. Moore, B. M. Moore: Voluntary preference for alcohol of white rats after gonadectomy.*

Nash, Joe B., G. A. Emerson, W. T. Moore and B. M. Moore: Effects of single fatty acids on voluntary alcohol preference of white rats.*

* Papers will be presented at the meeting of the American Society for Pharmacology and Experimental Therapeutics at Chicago in April, 1953. Abstracts are to be published in the Federation Proceedings.

d) Housekeeping Details. The extraordinary contributions of the several members of our staff, named above, to the progress of work under grant ONR-553(00)-NR-120-176 have been most heartening. In particular, Dr. Melvin Schadewald has served competently to broaden the scope of the problem and to add necessary morphologic controls; in this he has been aided by Marilyn Beavers, a technician, who has done most of the histologic work. W. T. Moore, who is paid a full-time salary from the grant, and C. Dugar, who received a small supplement from the grant, have worked satisfactorily during the past 6 months; Mrs. B. M. Moore has done the tedious chore of making daily observations on some 200 - 250 animals most faithfully. Three graduate students, Joe B. Nash, R. G. Brown and Nicholas Plotnikoff, have been active in various phases of the work, chiefly in cooperation with medical students who work part-time on single problems. No support from other sources has been received in direct support of this work, but a part of a grant received from The Stuart Company, Pasadena, California, in December, 1952, will probably be used to study effects of labile methyl donors on chronic preference in rats, although the chief problem of interest to The Stuart Company is along the lines of acute effects of these agents in alcoholic intoxication.

Sincerely yours,

George A. Emerson

George A. Emerson, Ph. D.
Professor of Pharmacology
and Toxicology

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ABSTRACTS

1. Effects of isonicotinyl hydrazine on smooth muscle. Robert G. Brown and G. A. Emerson. Constipation and delay in micturition are noted clinically with isonicotinyl hydrazine. Isolated ileum strips of rabbits, cats and dogs show no consistent response to 160 γ/ml. in Sollmann-Rademaeker's solution. 80 γ/ml. do not modify effects of acetylcholine, histamine or $BaCl_2$ on rabbit ileum. In the intact dog anesthetized with pentobarbital, a cumulative dose of 265 mg/Kg. produces clonic convulsions readily antidoted with pentobarbital, whereas lower doses produce no consistent effect upon intestinal motility, bladder tone, carotid pressure or respiration. Isonicotinyl hydrazine was administered to 5 rabbits, one hour after food and water were removed; 5 controls received water. After the technique of Eddy (J. Pharmacol. Exptl. Ther., 45: 339, 1932), passage of scybala was noted at 30-minute intervals for 8 hours. Food and water were made available for 1 hour, after 4 hours. 25 mg/Kg. p. o. significantly decreased the number of animals defecating, for 4 hours; 50 mg/Kg., for 8 hours. In 9 Sprague-Dawley rats fed a synthetic high-bulk diet, the weight of 24-hour fecal samples decreased markedly in every case after 50 mg/Kg. p. o. daily for 5 days; controls treated with water showed random changes. Lack of direct effects on smooth muscle, as confirmed and extended here, together with significant constipating effects in normal rats and rabbits, indicate a probable central mediation of the clinically observed side-effects.

2. Species variation in preference for alcohol and in effects of diet or drugs on this preference. G. A. Emerson, R. G. Brown, Joe B. Nash, W. T. Moore. Effects of deficient and corrected diets, vitamins, yeast and liver on voluntary alcohol intake of albino rats have been noted by others. Experience with related genera, comprising daily records of voluntary intake expressed as ml/Kg. of 10% ethanol and of preference for alcohol over some 3000 Peromyscus c. californicus-days, 3400 Cricetus auratus-days, 5500 Sigmodon h. hispidus-days and > 12000 Sprague-Dawley rat-days, indicates marked variation in response to certain factors. Pregnant hamsters (C.), uniformly in 12 instances, greatly decreased their voluntary intake of alcohol either shortly before or at delivery, and for several days thereafter. Hamsters are also most sensitive to changes in environmental temperature, showing an increase in extreme cold and a decrease in heat. Deer mice (P.) show little change following massive doses of progesterone, and a long delayed decrease questionably related to heroic treatment with estradiol benzoate; they also show remarkably slight response to large doses of K tellurite. Spleen and liver supplements to their diet have little effect. Cotton rats (S.) do not decrease their alcohol intake after prolonged supplementation of their diet with liver, and are resistant to effects of yeast. Together with the observed extremely high voluntary intake of 10% ethanol by P. and C. in contrast to that of S. and, usually, albino rats on an adequate diet, these findings may be taken to indicate a relation of metabolism to chronic "alcoholism" in animals and man, as postulated by Williams, but may also be interpreted in terms of behavioral differences between spp.

3. Effects of pregnancy and lactation on voluntary alcohol intake of hamsters. Joe W. Carver, Joe B. Nash, G. A. Emerson and W. T. Moore. Hamsters, given a choice between 10% V/V of 95% ethanol and water since weaning, usually show a marked drop in intake of alcohol at or 1 - 3 days before the end of gestation. Total volume of fluid ingested may also decrease significantly, so that preference for alcohol may not decrease proportionately to its intake. During lactation, both preference for alcohol and intake of alcohol usually decrease. Daily observations of alcoholic behavior of 50 hamsters are analyzed during periods of pregnancy and lactation. This study was supported in part by ONR grant 553-(00)-NR-120-178.

4. The Dose-Effect Relation: critique of estimation of mass of biological material. Charles D. Chamberlain, Jr., C. D. Leake and G. A. Emerson. Diurnal variations in body weight of immature, adult male, adult female and lactating hamsters given food ad lib. indicate that gross errors may result from estimations of quantitative effects of drugs expressed as administered mass of drug/mass of biological material, at different times of the day. Even larger variations are encountered in successive daily weights during pregnancy. Although the major cause of these variations may be related to differences in weight of the contents of the alimentary tract, other factors are also significant. Since absorption, distribution, detoxication and excretion of certain drugs may be drastically different in animals in the post-absorptive rather than the fed state, even short periods of inanition designed to minimize weight variations may significantly affect the dose-effect relation. In any species, validity of studies involving quantitative comparisons of activity of different agents is best if all agents are given under identical conditions, including the time of day. This study was supported in part by ONR grant 553-(00)-NR-120-178.

5. Sulfobromophthalein clearance in white rats after damage to the liver. Peter Y. Wong, C. D. Chamberlain, Jr., M. Schadewald, Joe B. Nash, G.A.Emerson. Rates of disappearance of 25 - 100 mg/Kg. of bromsulphalein Na after i.v. injection, from the plasma of healthy adult Sprague-Dawley rats, were estimated by a modification of the method of Casals and Olitsky (Proc. Soc. Exp. Biol. and Med., 63: 383, 1946) suggested by Mann and Lemonde (Rev. Canad. Biol., 10: 167, 1951). Normal mean rates are compared with mean clearance rates in partially hepatectomized rats, and rats receiving repeated gavages of large doses of ethanol, with and without supplementary pharmacologic insults to the liver. The effect of pre-treatment with compounds containing labile methyl groups is also noted. This study was supported in part by ONR grant 553(00)-NR-120-178.

6. Voluntary preference for alcohol of white rats after gonadectomy. Melvin Schadewald, G. A. Emerson, W. T. Moore and B. M. Moore. Among 40 Sprague-Dawley rats given a choice of 10% V/V of 95% ethanol and water for 55 days while on an adequate stock diet, the mean values for preference for alcohol, estimated daily as volume of 10% ethanol/total volume of fluid ingested, and for ml/Kg. of ethanol ingested showed a markedly higher preference and intake by males. This finding was uniform throughout this period except for the first 5 days. For the 46th-50th days, mean daily values for 22 females and 18 males, with their standard errors, were 12.6±1.7 and 30.1±3.9% for preference and 2.03±0.33 and 3.71±0.51 ml/Kg. of 95% ethanol, respectively. Five males with the highest intake and 5 females with the lowest intake were deprived of their gonads on the 55th day. During the next 35 days, daily estimations showed no significant changes in intake or preference, although both groups became more variable and a tendency for decreased intake in the males and increased intake in the females was apparent. Both groups were later treated with testosterone or estradiol. Results are interpreted in terms of persistence of steroid hormones after gonadectomy, anabolic factors unrelated to steroid hormones, and results of treatment of Peromyscus c. californicus previously reported. This study was supported in part by ONR grant 553-(00)-NR-120-178.

7. Effects of feeding single fatty acids on voluntary alcohol preference of white rats. Joe B. Nash, G. A. Emerson, W. T. Moore and B. M. Moore. Twenty-seven Sprague-Dawley rats, given a choice of 10% V/V of 95% ethanol and water for 55 days while on an adequate stock diet, were fed thereafter with the diet of Mardones and Onfray (Rev. Chilena Hig., 4: 293, 1942), which is deficient in the vit. B complex, except that autoclaved yeast was omitted, 10% cellulose powder was added, and the lipid component was substituted by 5% of even-numbered fatty acids from C_6 - C_{16}, 1-hexadecanol, or high (3°) or low m. p. fractions of cottonseed oil. I nos. of the pure fatty acids were essentially C. Three similar rats were fed the same diet without lipid. After 40 days of daily observation of alcohol intake, half the rats were treated orally with linoleic, linolenic or oleic acids as 10% solutions in decanoic acid. Results are expressed in relation to biochemorphic relations of the saturate fatty acids, and discussed in relation to concepts of Williams and Mardones on the role of unsaturate fatty acids. This study was supported by ONR grant 553(00)-NR-120-178.